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✓ 1. 5665539. 04 Oct 93; 09 Sep 97. Immuno-polymerase chain reaction system for antigen detection. Sano; Takeshi, et al. 435/6; 435/7.1 435/7.2 435/7.5 435/7.8. C12Q001/66.

┘ 2. AU 200112598 A WO 200131056 A2. Detecting antigens by real-time immuno-polymerase chain reaction. useful e.g. for detecting prion proteins. based on amplification of marker nucleic acid. EL MOUALIJ, B. et al. C12Q001/68 G01N033/50.

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L7: Entry 1 of 2

File: USPT

Sep 9, 1997

DOCUMENT-IDENTIFIER: US 5665539 A

TITLE: Immuno-polymerase chain reaction system for antigen detection

Brief Summary Text (6):

J. Clin. Microbiol., 28:1968 (1990) describes a system for detection of amplified Chlamydia trachomatis DNA from cervical specimens by fluorometric quantitation in an enzyme immunoassay (EIA) format, which includes a polymerase chain reaction.

Brief Summary Text (10):

(b) contacting a linker molecule having the ability to bispecifically bind to biotin and to immunoglobulin G, with a segment of biotinylated nucleic acid marker to form a second complex comprising the linker and biotinylated nucleic acid;

Detailed Description Text (3):

There are several novel features involved in this invention. First, a nucleic acid sequence is used as the marker for detection of antigen. Second, antibody-linker-biotinylated marker conjugates are used to attach a marker molecule to an antigen. Third, enzymatic steps such as a polymerase chain reaction are used to amplify signals for detection of specific antigens. Fourth, due to the specificity and efficiency of nucleic acid amplification, the detection sensitivity of the immuno-PCR technology is superior to that of any existing antigen detection system and the method is, in principle, able to detect a single antigen molecule. No method of such sensitivity is currently available. Fifth, a wider variety of antigens can be detected by the immuno-PCR than by other currently available antigen detection systems.

Detailed Description Text (5):

A streptavidin-protein A chimera previously synthesized and disclosed in co-pending applications identified above was used as the linker molecule. The chimera has two independent specific binding abilities. One is its binding to biotin, derived from the streptavidin moiety, and the other is its binding to the Fc portion of an immunoglobulin G (IgG) molecule, derived from the protein A moiety. This bifunctional specificity both for biotin and antibody allows the specific conjugation of any biotinylated nucleic acid molecule to antigen-antibody complexes. Other linker molecules, such as any protein, peptide, nucleic acid marker chemically cross-linked to antibodies, or biotinylated marker nucleic acid cross-linked to biotinylated antibodies by streptavidin or avidin may be also advantageously utilized.

Detailed Description Text (6):

In the current invention, a streptavidin-protein A chimera or any other linker that possesses tight and specific binding affinity both for biotin and immunoglobulin G was used to attach a biotinylated marker specifically to antigen-monoclonal antibody complexes that had been immobilized on microtiter plate wells. Next, a segment of the attached marker was amplified by PCR. Analysis of the PCR products by agarose gel electrophoresis after staining with ethidium bromide allowed as few as 580 antigen molecules (9.6.times.10.sup.-22 moles) to be readily and reproducibly detected. Direct comparison with enzyme-linked immunosorbent assays (ELISA) with the use of a chimera-biotinylated alkaline phosphatase conjugate demonstrated that approximately 10.sup.5 times enhancement in antigen detection sensitivity was obtained with the use of immuno-PCR. Given the enormous amplification capability and specificity of PCR, the current immuno-PCR technology has a sensitivity greater than that of any existing antigen detection system and, in principle, is sensitive enough to be applied to the detection of single antigen molecules.

Detailed Description Text (7):

One mode of the immuno-PCR technology of the current invention, in which a specific antibody-DNA conjugate is used to detect antigens, utilizes immobilization of various

amounts of an antigen on the surface of microtiter plate wells. For initial testing, bovine serum albumin (BSA) was used as the antigen because of the availability of both the pure protein and monoclonal antibodies against it. The detection procedure used was similar to conventional enzyme-linked immunosorbent assays (ELISA). Instead of an enzyme-conjugated secondary antibody directed against the primary antibody, as in typical ELISA, a biotinylated linear plasmid DNA (pUC19), conjugated to the streptavidin-protein A chimera, was targeted to the antigen-antibody complexes. A segment of the attached marker was amplified by PCR with appropriate primers and the resulting PCR products were analyzed by agarose gel electrophoresis after staining with the ethidium bromide.

Detailed Description Text (8):

The concept of immuno-PCR of the current invention is shown schematically in FIG. 1. By using linker molecule X which has bispecific binding affinity both for the marker for antibody, a molecule used as a marker can be specifically attached to an antibody-antigen complex. Marker molecules are typically DNA, RNA, DNA-RNA hybrids, their derivatives, fragments, segments or analogues. The attached marker allows the amplification of its segment(s) by PCR with appropriate primers. The enormous amplification capability and specificity of PCR allows the production of large amounts of specific DNA segments as PCR's products. These products can be detected by various methods known and used in molecular biology such as, for example, by agarose gel electrophoresis. The presence of specific PCR products demonstrates that marker molecules are attached to antigen-antibody complexes, indicating the presence of antigen. In addition, the quantitation of PCR products also provides the estimation of the number of antigens (epitope).

Detailed Description Text (11):

Linker molecule can be any material which is able to specifically recognize, or which possesses both the antibody-binding domain and also the biotin-binding domain. The linker may be any protein, peptide, nucleic acid marker cross-linked to antibody or biotinylated marker nucleic acid cross-linked to biotinylated antibody by streptavidin or avidin. The most preferred linker is a recombinant streptavidin-protein A chimeric protein.

Detailed Description Text (49):

Detection of Antigen Immobilized on Microtiter Plate by Immuno-PCR

Detailed Description Text (52):

Because of the availability of pure antigen and of monoclonal antibodies against it, to develop and to test the immuno-PCR method of this invention, bovine serum albumin (BSA) was used as the antigen. The procedures for detecting BSA immobilized on a microtiter plate by immuno-PCR are described below. The detection method and assays for other antigens are run under the same or slightly modified conditions.

Detailed Description Text (55):

Immuno-PCR technology using a specific DNA molecule as the marker can reliably detect very small amounts of antigen with sufficient reproducibility. The sensitivity of immuno-PCR is superior to that of any existing antigen detection system. Therefore, this technology is particularly useful for detecting rare antigen molecules in biological samples. The extremely high sensitivity of immuno-PCR enables this method to detect single antigen molecules; no current method allows detection with such a degree of sensitivity. The sensitivity of immuno-PCR can be controlled by varying one or more key factors. This suggests that immuno-PCR technology can be used on a wide variety of biological samples.

Detailed Description Text (63):

In principle, the immuno-PCR technology can be applied to detection of single molecules. No method is currently available which would have the same degree of sensitivity. In addition, the extremely high sensitivity of the immuno-PCR considerably reduces the amounts of antibodies required resulting in reduced assay costs. This is particularly useful when large amounts of specific antibodies are not available.

Detailed Description Text (64):

The sensitivity of immuno-PCR can be controlled by varying one or more key factors,

such as the number of amplification cycles and the detection methods for PCR products. This characteristic allows immuno-PCR technology to be applied to a wide range of biological samples, in which the number of antigens varies considerably.

Detailed Description Text (65):

Another advantage of immuno-PCR technology is its simplicity. Immuno-PCR does not include any complex procedures. Thus it allows the development of fully automated assay systems without a loss of sensitivity. This characteristic offers a great promise for applications in clinical diagnostics.

Detailed Description Text (68):

Using a similar configuration, any antigen molecule, which is efficiently separated from unbound antibody and unbound chimera-nucleic acid conjugates, can be detected by immuno-PCR without the need for modification of the basic procedure. Such antigen molecules include:

Detailed Description Text (75):

Pre-conjugation of nucleic acid makers to antibodies, i.e., use of antibody-chimera-nucleic acid marker conjugates, should decrease background levels of samples containing molecules that can bind protein A. The current invention is particularly useful for diagnostic purposes. The type and quantity of antigen present in the blood and other bodily fluids is detected by the assay of the current invention. The assay can also be useful for detection of immunodeficiencies allergies.

CLAIMS:

1. A method for detecting a rare ligand in a sample, said method comprising steps:

(1) contacting said sample with a conjugate comprising a non-nucleic acid receptor capable of specifically binding said rare ligand and a nucleic acid marker comprising a predetermined nucleotide sequence to form a specifically bound complex of said rare ligand and said conjugate;

(2) specifically detecting the presence of said nucleic acid maker of said complex,

wherein the presence of said nucleic acid marker indicates the presence of said rare ligand in said sample.

2. A method according to claim 1 wherein said specifically detecting step comprises amplifying said nucleic acid marker in a polymerase chain reaction.

4. A method according to claim 1 wherein said receptor is non-covalently conjugated to said nucleic acid marker by a bi-specific linker capable of specifically binding both said receptor and said nucleic acid marker.

5. A method according to claim 1 wherein said receptor is non-covalently conjugated to said nucleic acid marked by a bi-specific linker capable of specifically binding both said receptor and said nucleic acid marker, said nucleic acid marker comprises a hapten and said linker specifically binds said nucleic acid marker at said hapten.

6. A method according to claim 1 wherein said receptor is non-covalently conjugated to said nucleic acid marker by a bi-specific linker capable of specifically binding both said receptor and said nucleic acid marker, said nucleic acid marker comprises a hapten and said linker specifically binds said nucleic acid marker at said hapten, and said hapten is biotin.

13. A method for detecting a ligand in a sample, said method comprising steps:

(1) contacting a sample comprising a ligand with a conjugate comprising a non-nucleic acid receptor capable of specifically binding said ligand and a nucleic acid marker comprising a predetermined nucleotide sequence to form a specifically bound complex of said ligand and said conjugate, wherein said receptor is an antibody and said ligand is an antigen or hapten;

(2) specifically detecting the presence or absence of said nucleic acid marker of said complex, wherein said specifically detecting step comprises amplifying said nucleic acid marker in a polymerase chain reaction,

wherein the presence of said nucleic acid marker indicates the presence of said ligand in said sample.

14. A method according to claim 13 wherein said receptor is non-covalently conjugated to said nucleic acid marker by a bi-specific linker capable of specifically binding both said receptor and said nucleic acid marker.